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Ketogenesis and Malonyl Coenzyme A Content
of Liver from Streptococcus pneumoniae-infected Rats

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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ABSTRACT

Malonyl-CoA is a possible regulator of ketogenesis. Since infection partially inhibits starvation ketosis, studies were performed to determine if malonyl-CoA content was the limiting factor in ketogenesis during an infection. Malonyl-CoA was increased in fed rat liver and decreased in fasted and fasted-infected rat liver. This suggests that malonyl-CoA content does not regulate ketogenesis during an infection.

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Recent experiments by McGarry et al. (1977) have shown that hepatic malonyl-CoA reduces ketogenesis from oleic acid by inhibiting the carnitine acyltransferase I (palmitoyl-CoA:L-carnitine O-palmitoyltransferase, EC 2.3.1.21) enzyme which is thought to be the regulatory step in hepatic ketogenesis. Since hepatic concentrations of malonyl-CoA have been shown to be high in states of reduced ketogenesis, such as carbohydrate feeding and low in states such as starvation or fat feeding (Guynn et al., 1972), it seemed a likely regulatory factor during an infectious state.

It has previously been shown that ketone body concentration is partially decreased during a bacterial infection (Blackburn et al., 1973, Neufeld et al., 1976). Cook et al. (1978) have concluded that malonyl-CoA and/or the concentration of available long-chain fatty acids are the major determinants of the rate of ketogenesis. Although circulating free fatty acids are decreased during most infections, reduced availability cannot account for the marked reduction in the rate of ketogenesis which was observed when the isolated livers from Streptococcus pneumoniae-infected rats were perfused with oleic acid (Pace et al., 1977).

Therefore, malonyl-CoA was measured in livers from meal-fed, fasted, and fasted-infected rats. Our findings confirmed that hepatic malonyl-CoA content is high in the fed state and decreased during a fast, which is the reciprocal of ketone body concentration. However, this reciprocal relationship was not apparent during an infection, since both ketone body and malonyl-CoA concentrations were low.

Experimental

Male, Fisher-Dunning rats (F-344/Mai f, obtained from Microbiological Associates, Walkersville, Maryland, U.S.A.) weighing 175-200 g were meal-fed a standard diet (Teklad Test Diets, Division of ARS/Sprague-Dawley, Madison, Wisconsin, U.S.A.) (10% fat, 26% protein, 60% carbohydrate) between 0800 and 1000 hours. Rats were maintained in a light- and temperature-controlled room [12 h light (1000 to 2200 hours) and 12 h dark, $23^{\circ}\text{C} \pm 1$] during the experiment. After 2 weeks on this feeding schedule, 10 rats were killed at 1000 hours and the remainder were inoculated with either 10^4 live or heat-killed S. pneumoniae, type Ia 5, by subcutaneous injection in the nape of the neck. Food was withheld from both groups of rats, with those receiving the heat-killed organism serving as fasted controls. Details concerning the preparation of inoculated S. pneumoniae and clinical manifestations of the infection in rats have been published elsewhere (Wannemacher et al., 1971).

At 1000 hours, 24 h and 48 h post inoculation, 10 rats from each fasted group were stunned by a blow to the head and killed by cervical dislocation. The liver was removed and crushed between liquid nitrogen cooled aluminum blocks within 10 s. Blood which had accumulated in the chest cavity after severing the vena cava was collected in heparinized tubes.

Plasma ketone bodies (β -hydroxybutyrate and acetoacetate) were measured as described by McGarry et al. (1970). Hepatic ketones were measured as described previously (Neufeld et al., 1976). Malonyl-CoA was determined by a modification of the method of Guynn et al. (1972).

Free fatty acids were determined by the method of Dalton & Kowalski, (1967).

Values from meal-fed, 24 and 48 h fasted, and 24 and 48 h fasted-infected rats were compared based on one-way analysis of variance. Results are presented as mean \pm S.E.M. for 10 rats in each group.

Results and Discussion

The subcutaneous inoculation of 10^4 S. pneumoniae resulted in elevated rectal temperatures at 24 h; by 48 h the body temperature in the fasted-infected rats began to decline. Other clinical manifestations similar to those reported previously (Wannemacher et al., 1971) were observed in the infected rats by 24 h.

During this infection there was a decrease in the total plasma ketone bodies compared to concentrations in fasted controls (Table 1). This decrease did not appear to result solely from the reduced availability of fatty acids (Table 1), as isolated livers from S. pneumoniae-infected rats were previously shown to have a decreased ketogenesis from perfused long-chain fatty acid (Pace et al., 1977). It has also been reported that despite any decrease in their plasma concentration, the movement of free fatty acids into the liver continues (Fiser et al., 1974) and their incorporation into triglycerides is sometimes accelerated (Guckian, 1973; Fiser et al., 1974; Canonico et al., 1977).

Malonyl-CoA concentration was significantly decreased in the livers of fasted-control and fasted-infected rats when compared to meal-fed rats (Table 2). The effect of fasting on hepatic malonyl-CoA

concentrations is similar to values obtained by Guynn et al. (1972). As reported previously (Neufeld et al., 1976), hepatic ketone body concentrations were elevated in the fasted-control but were significantly lower in the fasted-infected rats. Although liver weight decreases during a fast and increases in infection, the changes in tissue content of malonyl-CoA and ketone bodies did not appear to vary with tissue mass (Table 2).

The first step specific for hepatic long-chain fatty acid oxidation is the carnitine acyltransferase reaction (McGarry & Foster, 1977). Although liver carnitine concentrations have been shown to correlate with hepatic ketogenic capacity during fasting (McGarry et al., 1975; Pace et al., 1978) and diabetes (McGarry et al., 1975), no such relationship was observed during bacterial infection (Pace et al., 1978).

McGarry et al. (1977) have suggested that malonyl-CoA acted as a suppressor of fatty acid oxidation by inhibiting the carnitine acyltransferase I enzyme. In the present studies a reciprocal relationship was observed between malonyl-CoA concentration and plasma and hepatic ketone body concentrations in the fed and fasted groups; however, whereas ketone bodies decreased during infection, malonyl-CoA remained at the low concentrations typical of fasting.

In the meal-fed rat host, insulin appears to block the action of glucagon, thus increasing the hepatic concentration of malonyl-CoA and lowering that of carnitine (Pace et al., 1977). In contrast, the fasted-infected rat host has elevated peripheral and portal vein concentrations of insulin and glucagon, with a reduced ratio of insulin

to glucagon (Zenser et al., 1974). This ratio change should result in a decrease in hepatic malonyl-CoA, elevated carnitine concentrations and rate of ketogenesis. While the concentration level of hepatic malonyl-CoA is decreased and that of carnitine is increased (Pace et al., 1977), the rate of ketogenesis is markedly reduced as compared to fasted controls. These observations suggest that infection inhibits the rate of hepatic ketogenesis by a mechanism other than that observed in the meal-fed host.

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Table 1. Plasma free fatty acid and ketone body concentrations in meal-fed, fasted and Streptococcus pneumoniae-infected rats

Plasma free fatty acids and ketone bodies were determined as described in the Experimental Section. The results are expressed as means \pm S.E.M. of 10 rats in each group; * † indicate $P < 0.01$ for comparison against meal-fed and fasted controls, respectively.

Condition	Plasma free fatty acid (mEq/l)	Plasma ketone bodies ($\mu\text{mol/ml}$)		
		Acetoacetate	β -Hydroxybutyrate	Total
Fed	503 ± 104	0.17 ± 0.05	0.66 ± 0.06	0.83 ± 0.06
Fasted 24 h	$901 \pm 74^*$	$0.53 \pm 0.04^*$	$1.48 \pm 0.11^*$	$2.01 \pm 0.14^*$
Fasted 48 h	$1164 \pm 99^*$	$0.76 \pm 0.04^*$	$3.42 \pm 0.16^*$	$4.18 \pm 0.19^*$
Fasted-Infected 24 h	$710 \pm 65^*$	$0.54 \pm 0.05^*$	0.89 ± 0.13	$1.43 \pm 0.15^{*+}$
Fasted-Infected 48 h	$444 \pm 64^{\dagger}$	$0.39 \pm 0.03^{*+}$	$1.32 \pm 0.12^{*+}$	$1.71 \pm 0.14^{*+}$

Table 2. Malonyl-CoA and ketone body concentrations in meal-fed, fasted and Streptococcus pneumoniae-infected rat liver

Livers were extracted and analyzed as described in the Experimental Section. The results are expressed as means \pm S.E.M. of 10 rats in each group; * \dagger indicate $P < 0.01$ for comparison against meal-fed and fasted controls, respectively.

Condition	Malonyl-CoA		Ketone bodies	
	(nmol/g)	(nmol/total liver)	(μ mol/g)	(μ mol/total liver)
Fed	56.6 \pm 7.8	493.2 \pm 66.8	0.26 \pm 0.01	2.3 \pm 0.14
Fasted 24 h	33.1 \pm 3.5*	273.2 \pm 30.9*	1.03 \pm 0.10*	8.56 \pm 0.93*
Fasted 48 h	35.6 \pm 2.9*	231.3 \pm 24.7*	1.85 \pm 0.06*	11.87 \pm 0.51*
Fasted-Infected 24 h	37.5 \pm 2.8*	276.8 \pm 18.5*	0.84 \pm 0.09*	6.38 \pm 0.56* \dagger
Fasted-Infected 48 h	37.9 \pm 3.7*	316.2 \pm 32.8*	0.51 \pm 0.03* \dagger	4.24 \pm 0.26* \dagger